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Running head: Herbivore impacts on tree health

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Abstract

Forests make up a large portion of terrestrial plant biomass, and the long-lived woody plants that dominate them possess an array of traits that deter consumption by forest pests. Although often extremely effective against native consumers, invasive species that avoid or overcome these defenses can wreak havoc on trees and surrounding ecosystems. This is especially true when multiple invasive species co-occur, since interactions between invasive herbivores may yield non-additive effects on the host. While the threat posed by invasive forest pests is well known, long-term field experiments are necessary to explore these consumer-host interactions at appropriate spatial and temporal scales. Moreover, it is important to measure multiple variables to get a 'whole-plant' picture of their combined impact. We report the results of a four-year field experiment addressing the individual and combined impacts of two invasive herbivores, the hemlock woolly adelgid (*Adelges tsugae*) and elongate hemlock scale (*Fiorinia externa*), on native eastern hemlock (*Tsuga canadensis*) in southern New England. In 2011, we planted 200 hemlock saplings into a temperate forest understory and experimentally manipulated the presence/absence of both herbivore species; in 2015, we harvested the 88 remaining saplings and assessed plant physiology, growth, and resource allocation. Adelgids strongly affected hemlock growth: infested saplings had lower above/belowground biomass ratios, more needle loss, and produced fewer new needles than control saplings. Hemlock scale did not alter plant biomass allocation or growth, and its co-occurrence did not alter the impact of adelgid. While both adelgid and scale impacted the concentrations of primary metabolites, adelgid effects were more pronounced. Adelgid feeding simultaneously increased free amino acids local to feeding sites and a ~30% reduction in starch. The cumulative impact of adelgid-induced needle loss, manipulation of nitrogen pools, and the loss of stored resources likely accelerates host decline

through disruption of homeostatic source-sink dynamics occurring at the whole-plant level. Our research stresses the importance of considering long-term impacts to predict how plants will cope with contemporary pressures experienced in disturbed forests.

Keywords: exotic species invasions, forest understory, herbivory, piercing-sucking insects, plant resource allocation, plant primary metabolism, *Tsuga canadensis*

Introduction

Forests make up a large fraction of terrestrial plant biomass and provide a wide variety of ecologically- and economically-important ecosystem functions. The long-lived woody plants that dominate these systems possess a formidable array of both constitutive and inducible defenses against exploitation (Coley et al. 1985). While plant-consumer coevolution selects for defenses effective against native exploiters, it may not protect against newly-arrived consumers with novel feeding modes or attack strategies. In such situations, the mismatch in generation time between long-lived woody plants and their consumers may prove catastrophic: invasive species have driven multiple tree species to functional extinction (Boyd et al. 2013).

The threat posed by invasive species is particularly acute in temperate forests (Lovett et al. 2006, Gandhi and Herms 2010). These regions have relatively low family-level woody plant diversity and are dominated by a small number of tree species. In addition, global transportation networks linking formerly-disjunct temperate regions have sharply increased the potential for, and number of, species invasions (Lovett et al. 2006, Gandhi and Herms 2010). As a result, many temperate tree species are now forced to contend with multiple invasive species as well as their native consumers. The cumulative impact of multiple herbivores is rarely additive, with the

outcome often depending on the sequence of attack (Ali and Agrawal 2014) and the feeding guild of the insect (Zvereva et al. 2010). Such non-additive effects are particularly likely when early-arriving herbivores induce changes in the host plant (Fournier et al. 2006, Morris et al. 2007, Pieterse and Dicke 2007, Stam et al. 2014) that alter the impact of later-arriving species (Wallin and Raffa 2001, Soler et al. 2012).

There are two key mechanisms by which herbivores impact plants. They can alter performance traits (growth, reproduction and survival) of the host and/or they can induce local and systemic changes in plant chemistry. Both mechanisms may affect the susceptibility, resistance or tolerance of plants to subsequent attack and can mediate subsequent interactions among herbivores (Denno et al. 1995, van Zandt and Agrawal 2004, Viswanathan et al. 2005). For example, reductions in foliar nutrients or changes in defensive chemistry following damage are well known to affect the suitability of hosts for late-arriving herbivores, with consequences for growth and survival (McClure 1980, Inbar et al. 1999, Soler et al. 2007). These changes may magnify the impact of one or both herbivores, leading to invasional meltdown (Simberloff and Von Holle 1999); alternately, they can decrease the cumulative impact and generate invasional interference (Yang et al. 2011, Rauschert and Shea 2012). Understanding what factors determine the outcome of herbivore interactions on a shared host is especially important for sap-feeders, a group whose impact on plant fitness can equal or exceed that of defoliators (Zvereva et al. 2010). Given these plant-wide effects, a 'whole-plant' analysis of herbivore-induced changes is required.

Work addressing forest pest invasions generally takes one of two approaches. Examining pests at the forest scale provides important data on long-term trends in plant health and pest densities, but the logistical constraints inherent in such large-scale and long-term research means that such work is rarely experimental (Preisser et al. 2008). This is important since studies

93 comparing naturally-infested and herbivore-free trees in order to assess herbivore impacts (e.g.,
94 Domec et al. 2013) conflate cause and effect and cannot be used to quantify non-additive effects
95 (Nykänen and Koricheva 2004). Conversely, efforts addressing the impact of pests on plant
96 physiology or chemistry are often short-term (i.e., <1 year in duration) and examine a subset of
97 plant traits. The latter type of study are also often conducted in relatively controlled settings
98 (e.g., greenhouses or plantations) whose abiotic conditions may differ markedly from natural
99 systems (e.g., Miller-Pierce et al. 2010). While great strides have been made using both
100 approaches, understanding some aspects of forest invasions may require *in situ* field experiments
101 that are conducted at system-appropriate temporal/spatial scales and measure a wide array of
102 plant traits in order to produce a 'whole-organism' picture.

103 Regardless of approach, relatively little work on forest pests has addressed their impact
104 on the ontogenetic stages necessary for stand regeneration and succession. Because seedlings and
105 saplings can live for decades in the low-light forest understory, their responses to herbivory may
106 not match those of mature trees (Boege and Marquis 2005, Barton and Koricheva 2010). For
107 example, understory saplings that rely on early-spring carbon acquisition prior to canopy leaf-out
108 (Hadley and Schedlbauer 2002, Polgar and Primack 2011) may be especially harmed by
109 decreased photosynthesis following attack. Such impacts may influence resource allocation
110 trade-offs and alter plant functional priorities concerning growth, resource acquisition and
111 herbivore defense (Boege and Marquis 2005).

112 We aim to examine the complex ways in which multiple herbivores impact the
113 physiology and growth of a long lived woody plant. In order to address this, we utilize a large-
114 scale and long-term field experiment. This unique design examines the individual and combined
115 impacts of two invasive herbivores, the hemlock woolly adelgid (*Adelges tsugae*) and elongate

hemlock scale (*Fiorina externa*), on the growth, physiology and chemistry of eastern hemlock (*Tsuga canadensis*, 'hemlock') understory saplings. The two pests co-occur in a portion of their ranges – especially in southern New England, New York, and Pennsylvania. This co-occurrence has become more pronounced over the past three decades as the ranges have shifted (see appendix S1, 'Natural History of the System', for additional details). In 2011, we planted several hundred hemlocks into a deciduous forest understory in southern New England (USA) and inoculated them individually, simultaneously, or sequentially with one, both, or neither herbivore over a four-year period. In 2015, we harvested the hemlocks and quantified multiple aspects of growth, metabolism, and resource allocation in both above- and below-ground tissue. Our 'whole-tree' results reveal the disparate impact of these two herbivores and the complex ways in which herbivory alters woody plant growth and physiology.

Materials and Methods

In April 2011, 200 ~0.3 m tall hemlock saplings (Van Pines Nursery, West Olive, MI, USA) were planted into a hardwood (maple/oak dominated) forest at the Kingston Wildlife Research Station (Kingston, RI). The trees had not been treated with insecticide. Saplings were planted in a 10 x 20 grid ~1.25 m from each other; initial heights and basal diameters were recorded prior to planting. Each sapling was enclosed in a mesh-covered (Agribon-15, Johnny's Selected Seeds, Waterville, ME, USA; 90% light) wire cage to exclude deer browsing and prevent cross-treatment contamination. The mesh bags were removed between December and March, while both insects are immobile, to prevent snow from collapsing the cages.

Following planting, each tree was randomly assigned to an herbivore treatment (Table 1).

Inter-plant adelgid and scale dispersal is most likely to occur prior to spring leaf-out, when both sub-canopy wind velocities and crawler densities are high (McClure 1989). Each spring, we simulated yearly dispersal by inoculating each tree with foliage infested with the appropriate insect; herbivore-free trees were 'inoculated' with uninfested foliage. Herbivore-infested foliage was collected from singly-infested stands previously identified in surveys (Gómez et al. 2015). Inoculations were conducted using a standard protocol (Butin et al. 2007); because adelgid emerges earlier than EHS, inoculations were conducted in May and June, respectively.

Starting in 2011, trees in three treatments were annually inoculated with adelgid ('HWA') only, scale ('EHS') only, both, or neither (=uninfested foliage) insects for four years (HWA-4, EHS-4, and Both-4, respectively). Starting in 2013, some adelgid-only and some scale-only trees were thereafter annually inoculated with both insects, creating two 'priority effect' treatments (HWA→Both, EHS→Both). In 2013, we also began annual inoculations of previously-uninfested trees with adelgid-only, scale-only, or both insects for two years (HWA-2, EHS-2, Both-2). A subset of trees remained herbivore-free throughout (Control; Table 1).

Insect densities were assessed twice yearly, in early spring and late fall, throughout the experiment. Details regarding insect densities from 2011-2014 are presented elsewhere (Schaeffer et al. 2017). Because our focus is on cumulative treatment impacts, we report November 2014 insect densities solely as an indicator of whole-tree infestation levels. In fall 2014, insect densities on newly-produced foliage were similar for adelgid (2.01 ± 0.18 [SE] insects cm^{-1}) and scale (1.99 ± 0.26 insects cm^{-1}) (Table 1). These infestation levels fall within those observed in the field and in prior studies where hemlock trees were experimentally inoculated (Miller-Pierce et al. 2010, Soltis et al. 2015). As in prior work, the densities of both adelgid and scale were higher in single-species treatments than when they co-occurred (150%

higher for adelgid and 50% higher for scale), suggesting plant-mediated interference competition between these two herbivores (Preisser and Elkinton 2008).

Between 2011-2015, we lost replicates to Hurricane Sandy, cross-treatment contamination, browsing by white-tailed deer (*Odocoileus virginianus*), and isolated outbreaks of secondary pests (e.g., *Oligonychus ununguis* mites and *Nucalaspis* sp. scales). There were several trees in the single-herbivore treatments (i.e., treatments EHS-2, EHS-4, HWA-2, and HWA-4) whose low insect densities (<0.5 insects/cm; the bottom 15% of fall 2014 densities) may have obscured the impact of insect damage; we excluded these trees from the harvest. The 88 remaining trees were intensively monitored in early spring prior to the May 2015 harvest.

Spring monitoring and harvest. In early April 2015, three branches per tree were selected and marked. Between 15-19 April (prior to bud break and crawler emergence), these branches were used to quantify herbivore abundance and photosynthetic rates. Because insects were located on older needles, we calculated insect densities per marked branch by dividing the number of insects by $\geq$1-year needle biomass (insects g^{-1} DW). We chose this metric because (1) adelgid settles at the needle base while scale settles on the needles; and (2) similarly-sized branches could vary in needle density (C. Wilson, *personal observation*). As a result, expressing density on a per-gram basis provided a more ecologically-relevant density metric in this case.

Photosynthetic rates were measured between 0800 and 1200 using one-year-old (2014 growth) foliage on the terminal end of each marked branch using a CIRAS-2 portable photosynthesis system (PP systems, Haverhill, MA, USA) with a 2.5 cm^2 cuvette and a CIRAS-2 LED light source of 1,500 μmol m^{-2} s^{-1}, a CO_2 concentration of 390 ppm, air flow rate at 350 cm^3 s^{-1}, and leaf temperature of 25°C. After each measurement, the foliage was photographed and ImageJ 1.44 (Abramoff et al. 2004) used to quantify needle area.

185 The 88 experimental trees were harvested over a 14-day period in May 2015. The time
186 and effort required for whole-tree excavation required us to split the trees into 22 four-tree
187 harvest groups, with each treatment represented in at least every third group; 1-3 groups were
188 harvested daily. Data on the timing of bud break is presented elsewhere, along with similar data
189 from another multi-year experiment (Whitney et al in preparation); bud break data in this paper
190 is used solely to calculate new flush production.

191 *Whole-plant biomass distribution*. Immediately prior to harvesting each tree, we recorded
192 its height and trunk diameter five cm above the root ball. Each marked branch was then clipped
193 at the base and placed on ice in a plastic bag. To ensure that we obtained a sufficient amount of
194 plant material for chemical analyses, we collected an additional randomly-selected branch from
195 each tree; all four branches were immediately transported to the laboratory for processing
196 (detailed below). The trunk of each tree was then clipped five cm above the root ball and the
197 aboveground portion dried for 24 hrs at 60^{o} C. We sorted dry material into three classes (new
198 flush, $\geq$ 1-yr needles, and wood). After the aboveground portion of each tree had been removed,
199 its root ball was excavated, cleaned of all dirt and foreign objects, dried as above, and weighed.
200 Belowground harvest and processing protocols are detailed elsewhere (Schaeffer et al. 2017).

201 *Chemical analyses.* In the laboratory, all insects on marked branches were removed using
202 a dissecting scope to avoid damaging any hemlock tissue. Each branch was separated into five
203 tissue types (new flush, 1-yr old needles, >1-yr old needles, 1-yr old stems, and >1-yr old stems)
204 and weighed; the fresh mass of each tissue was converted to dry mass using tissue-type-specific
205 conversion factors generated in a pilot experiment (Appendix S2: Table S1). Each type was kept
206 separate for each tree, stored at -20°C before being dried at -55°C for 72 hrs in a lyophilizer, then
207 ground into powder using a KLECO ball mill (Garcia Machines, Visalia, CA, USA).

Carbon (C) and nitrogen (N) content were determined by dry-combusting 2–3 mg of finely-ground material with a CHNOS analyzer (vario Micro cube, Elementar Americas, Mt. Laurel, NJ, USA). Starch was quantified using an EnzyChrom™ starch assay kit (BioAssay Systems, Hayward, CA, USA) as per manufacturer protocols. Briefly, 10 mg of root powder was boiled in one mL distilled water for five min, then centrifuged at 10,000 x g for two min; the supernatant containing soluble starch was set aside. The remaining pellet was reconstituted in 0.2 mL dimethyl sulfoxide and boiled for five min to obtain recalcitrant starch. The supernatants were combined and tissue starch levels (mg g^{-1} DW) determined using the kit.

We quantified free tissue amino acid levels and relative composition of individual amino acids following the protocol of Gomez et al. (2012). For each needle tissue type, 0.2 g of sample material was extracted in one mL 80% ethanol (v:v) at room temperature for 20 min. Samples were vortexed periodically and then centrifuged at 10,000 x g for ten min at room temperature. The supernatant was filtered through a 0.45 µm Acrodisk Syringe filter (Pall Gelman Laboratory, Ann Arbor, MI, USA), and the filtrate used for free amino acid determination using a commercial EZ: Faast™ kit (Phenomenex, Torrence, CA, USA) and GC-FID (Agilent Technologies, Waldbron, Germany) as per manufacturer protocols. Briefly, two µL of sample was injected (15:1 split) on a Zebron ZB-AAA column (0.25 mm x 10 m; Phenomenex) with the injector temperature set to 250°C. Helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1}. The initial oven temperature was set to 110°C, increased at a linear rate of 32° min^{-1} to 320°C, and held at 320°C for three min. This kit identifies and quantifies 22 amino acids; individual amino acids were identified by comparing retention times to amino acid standard solutions (norvaline as internal standard) and quantified using ChemStation software (Rev. B.04.02; Agilent Technologies, Waldbron, Germany).

Statistical analyses. All analyses were performed using R v. 3.2.2 (RCoreTeam 2014). Welch's *t*-tests were used to compare insect densities. We fit linear mixed-effects models and used a backward-model-selection approach to examine how the individual and interactive effects of adelgid and scale on hemlock. Adelgid and scale were treated as fixed factors, each with three levels corresponding to the length of infestation (0, 2, or 4 years) and an interactive term (HWA*EHS). Full and reduced models were ranked and compared based on Bayesian Information Criterion (BIC) values, a standard criterion for model selection. Details of each model, including the random effects used for each, are contained in Appendix S3. The *lme4* package was used to generate and compare models (Pinheiro et al. 2014). We used this approach to examine the individual and combined impact of adelgid and scale, as well as how herbivore-specific priority effects, affected the following: final height, final basal diameter, total biomass, aboveground biomass, belowground biomass, above-/belowground biomass ratio, needle/woody biomass ratio, new flush production, and photosynthesis.

Because tissue type and age can impact plant chemistry, we analyzed percent C, percent N, C:N ratio, total amino acids, and total starch using a modified approach. For stem and needle tissue, tissue age (1-year or >1-year) was included in the models. Because we did not have enough 1-year tissue to conduct a full suite of chemical analyses on it, our analyses of 1-year tissue are limited to percent C, percent N, and C:N ratio. We ran linear mixed-effects models with row and harvest date as random effects.

For amino acid analyses, 1-year and > 1-year needles were analyzed separately. To assess the effect of adelgid on amino acid levels, individual amino acids that were detected in <20% of all biological replicates and constituted <1% of the total amino acids ($\mu g\ g^{-1}$ DW) were removed from the datasets in order to prevent their over-influence in the analysis of profiles. The detection

of these amino acids followed no pattern with regards to treatment effects (logistic regressions; $P > 0.05$). For 1-year needles, these were: alpha-aminobutyric acid (ABA; detected in 3%), beta-aminoisobutyric acid (BAiB; detected in 19%), ornithine (ORN; detected in 13%), and sarcosine (SAR; detected in 2%), and for > 1-year needles, these amino acids were: alpha-aminobutyric acid (ABA; detected in 9%), ornithine (ORN; detected in 5%), and sarcosine (SAR; detected in 10%). For the remaining amino acids, tissue levels (μg g^{-1} DW) were Hellinger-transformed to normalize data on a total μg amino acid basis; transformed values were used in profile analyses.

Treatment differences in amino acid profiles were visualized with NMDS using the 'Bray-Curtis' dissimilarity index and the 'vegan' package (Oksanen et al. 2013) in R. This index was chosen because it consistently gave the highest rank-order similarity of all possible dissimilarity indices available in the 'vegan' package that account for amino acid abundance, and fitted the NMDS model with the lowest stress statistic (< 0.2 for all ordinations). The effect of adelgid, scale, and their interaction on needle amino acid profiles was assessed via PERMANOVA in the 'vegan' package with 10,000 permutations (Lieurance et al. 2015).

The effect of adelgid and scale on total amino acids was assessed via ANOVA followed by a post-hoc Tukey test. The influence of adelgid and scale infestation on individual amino acids was evaluated by first normalizing μg amino acid per mg total amino acids per g dry tissue mass (μg mg^{-1} g^{-1} DW), and then fitting an ANOVA model with adelgid infestation as the predictor; scale and the HWA*EHS interaction were removed from all regressions because neither influenced amino acid levels. The Benjamini-Hochberg false discovery rate-controlling procedure (Benjamini and Hochberg 1995) was used to correct for multiple comparisons.

Results

Growth and biomass allocation. Adelgids altered hemlock growth (i.e., final

measurements with initial values, when significant, present as a covariate) and biomass allocation; scales did not. There were no significant priority effects (i.e., prior colonization by one species did not affect the impact of the later-arriving species), and the HWA*EHS interaction was never significant. Because of this, we report only the main insect effects in the text (see Appendix S3 for full model outputs). Although neither insect affected total, above-, or below-ground plant biomass, adelgid altered plant biomass allocation (Fig. 1; Appendix S3: Tables S1 and S2). The above-/below-ground biomass ratio of adelgid-infested trees was 17% lower than adelgid-free trees ($F_{2,79}=6.62$, $P=0.01$; Fig. 1a) and the aboveground needle/woody biomass ratio was 16% lower in adelgid-infested trees ($F_{2,78}=4.53$, $P=0.01$; Fig. 1c). Adelgid-infested trees were also 7% shorter than adelgid-free trees ($F_{2,78}=3.67$, $P=0.03$), but did not differ in final basal diameter (Appendix S3: Table S1).

Early spring growth and photosynthesis. New flush production (grams/day) prior to harvest was ~30% lower for adelgid-infested versus adelgid-free trees ($F_{2,77}=36.54$, $P<0.001$; Fig. 2a), but was not reduced by scale ($F_{2,77}=1.90$, $P=0.16$; Fig. 2b).

There were no significant treatment-level effects of adelgid or scale on photosynthetic rates of 1-year-old foliage (Fig. 3a; Appendix S3: Table S3). Although there was no relationship between adelgid density and photosynthetic rates (Appendix S3: Table S4), scale density was negatively correlated with photosynthetic rates in trees infested with scale for two and four years (Appendix S3: Table S5).

Foliar chemistry. While adelgid substantially altered multiple aspects of foliar chemistry, scale had no significant impact. The N content of 1-year needles in adelgid-infested trees was 10% higher than in adelgid-free trees ($F_{2,166} = 10.80$, $P < 0.001$; Fig. 3b; Appendix S3: Table S7). Among trees infested with adelgid for two years, adelgid density was correlated with

300 percent N; this was not, however, the case among trees infested for four years with adelgid
301 (Appendix S3: Table S4). New-flush needles on adelgid -infested trees also had higher N levels
302 ($F_{2,69} = 4.22$, $P = 0.02$; Fig. 3b). Although starch concentration in 1-year needles was similar in
303 adelgid-free trees and trees infested with adelgid for two years, the starch content of trees
304 infested with adelgid for four was ~30% less than that of the other treatments (Fig. 3c). Scale
305 feeding increased starch in 1-year needles ($F_{2,155} = 3.95$, $P = 0.02$; Appendix S3: Table S8),
306 although total starch concentration was correlated with neither adelgid nor scale density
307 (Appendix S3: Tables S4 and S5).

308 Adelgid infestation altered the amino acid profiles (PERMANOVA; $P < 0.0001$) of both
309 1- and >1-year needles (Fig. 4a, 1-year needles; Fig. 4b, >1-year needles), while scale did not
310 (PERMANOVA; $P > 0.80$ for both). Valine, proline, isoleucine, and tryptophan were the major
311 drivers for both tissue types (Table 2a and 2b). Total free amino acid levels were greater in
312 adelgid-infested foliage for both 1-year (ANOVA; $F_{2,83} = 7.87$, $P < 0.001$; Table 2a) and >1-year
313 needles (ANOVA; $F_{2,83} = 11.90$, $P < 0.001$; Table 2b) vs. non-infested trees, a difference driven
314 primarily by proline. Two- and four-year infested foliage were not, however, significantly
315 different (post-hoc Tukey HSD).

316 **Discussion**

317 Exotic insects are an imposing force on native trees and their associated communities
318 (Lovett et al. 2006). Despite this, and the fact that native hosts often face attack by multiple
319 exotic herbivores (Gandhi and Herms 2010), *in situ* experimental evidence of the overall
320 consequences of attack for susceptible native species remains rare (Preisser et al. 2008). Our
321 multi-year manipulative study on woody plants growing in a natural setting provides a 'whole-
322 plant' perspective on how multiple invasive herbivores affect the growth and chemistry of a

naïve native tree. We found that chronic herbivory by two invasive piercing-sucking herbivores had divergent impacts on the growth and chemistry of their shared host, a foundational tree species in the temperate forests of the eastern United States (Ellison et al. 2005).

While multiple years of adelgid herbivory altered patterns of biomass allocation and primary metabolism in understory hemlock saplings, scale had minimal impacts. Although adelgid densities were lower when they co-occurred with scale ($t = 46.93$, $P < 0.01$; Table 1), neither prior nor simultaneous inoculation of hemlock saplings with scale altered the impact of adelgid on these understory plants. In general, dually-infested trees showed changes in allocation and metabolites typical of adelgid-only treatments. One possible explanation for the subdued effect of the scale is the presence of native armored scale (*Abgrallaspis ithacae)* on eastern hemlock. No native adelgid species attacks eastern hemlock. Since the introduction of the elongate hemlock scale did not present an entirely novel challenge to the host, the host may already have some existing defenses.

Plant growth and biomass distribution. Several years of adelgid infestation on hemlock saplings lowered above-/belowground and needle/woody tissue ratios. This is consistent with previous work (Soltis et al. 2014, Soltis et al. 2015), and was likely driven by a combination of reduced new foliar growth and premature needle desiccation/ loss. Our findings contrast with research on other plant species that respond to aboveground herbivory by shifting resources away from herbivore feeding sites and into stem and root storage sites (Babst et al. 2005, Babst et al. 2008). Despite adelgid-infested and adelgid-free trees having similar per-needle photosynthetic rates, the reduced production of new foliage, likely in combination with the loss of old needles, clearly hampers resource uptake in a light-limited environment. In turn, this restriction affected the production and allocation of primary metabolites in stems and needles.

346 *Primary metabolites*. Adelgid impacts on hemlock health were further reflected through
347 changes in primary metabolites. Herbivore-attacked plants often protect themselves via induced
348 changes in primary and secondary metabolism (Stam et al. 2014, Zhou et al. 2015), although
349 research to date has primarily addressed impacts of herbivory on secondary rather than primary
350 metabolism (Zhou et al. 2015). Our results also confirm previous work (Gómez et al. 2012)
351 showing that adelgids cause localized increases of N and the amino acid proline at their feeding
352 sites. Proline accumulation is a common plant response to drought stress (Delauney and Verma
353 1993); this and other adelgid-induced changes in hemlock physiology (Radville et al. 2011,
354 Domec et al. 2013) suggest that adelgid likely induces drought-like stress in its native host plant
355 (Gómez et al. 2012). For instance, paralleling increases in proline, adelgid-infested tissues had
356 lower levels of the amino acids isoleucine and tryptophan. A similar pattern has been observed in
357 *Arabidopsis* following aphid feeding. In *Arabidopsis* this pattern is associated with aphid-
358 induced increases in the hormone abscisic acid (ABA; Hillwig et al. 2016): adelgid also induces
359 ABA production following attack (Schaeffer et al. 2017). Although ABA induction is often
360 associated with water stress (Lee and Luan 2012), its induction may benefit piercing-sucking
361 insects via its antagonistic interactions with jasmonic acid (JA) signaling (Erb et al. 2009, Vos et
362 al. 2013), a key pathway for anti-herbivore defense. We hypothesize that ABA induction
363 following adelgid feeding aids its success through prevention of effective JA pathway signaling,
364 which is known to deter HWA crawlers (Schaeffer et al. 2017)
365 Starch is another key primary metabolite which plays an essential role in plant tolerance
366 to damage. Following herbivory, stored carbohydrates are frequently broken down and
367 remobilized to compensate for tissue loss (Appel et al. 2014). The post-attack mobilization of
368 these resources can benefit the host by fueling repair and regrowth (Trumble et al. 1993). Some

herbivores, particularly piercing-sucking insects, exploit hosts and stored resources via extra-oral digestion of stored carbohydrates like starch. This extra-oral digestion is achieved via deployment of salivary enzymes like α-amylase to local feeding sites. Adelgid, a piercing-sucking herbivore, has been hypothesized to use a similar feeding strategy (Oten et al. 2014). Our findings support this hypothesis: adelgid feeding for four years led to a ~30% reduction in starch levels in 1-year needles (Fig. 3c). The loss of stored resources through feeding, combined with loss of source tissues, likely accelerates host decline through disruption of homeostatic source-sink dynamics occurring at the whole-plant level.

Perspective on the impacts of multiple invasive herbivores across space and time. Plant stresses, especially when experienced during early ontogenetic stages, strongly affect resource allocation trade-offs concerning growth, resistance, storage, and reproduction (Boege and Marquis 2005). Understanding such trade-offs requires studies conducted at the appropriate temporal and spatial scales. Despite the lack of interference between adelgid and scale on any metric of hemlock health in this experiment, our observation of suppressed adelgid densities when co-occurring with scale (Table 1; also see Schaeffer et al. 2017), combined with multiple years of landscape-level observations (Gómez et al. 2015), suggests that the impact of scale on adelgid may be density-dependent and will likely become more pronounced on the landscape over time. Prior work in this system has found that higher densities of scale can significantly reduce adelgid densities and benefit the native host (Preisser and Elkinton 2008). Moreover, while adelgid densities have generally declined in our region of study over time, scale densities have steadily increased, effectively making scale the most abundant hemlock herbivore throughout much of New England (Gomez et al. 2015, Schliep et al. 2018). As scale abundance continues to increase, we predict that interference competition between these two herbivores

could buffer future declines of this foundational forest species in southern New England – where the ranges of the two pests overlap most prominently. While this may facilitate hemlock recovery in the northern portion of the invaded range, the impact on hemlock decline in the mid-Atlantic portion of the United States requires further study. Less certain, however, is how the two pests will interact with the shifting range of their host (McAvoy et al. 2017, Rogers et al. 2017).

In conclusion, we found that two invasive herbivores from the same feeding guild have disparate effects on biomass allocation, growth, and primary metabolism of an early ontogenetic stage of a foundational forest species. Our research stresses the importance of considering long-term impacts for predicting woody plant responses to contemporary pressures experienced in disturbed forests, especially in the case of life-stages that will dictate their future prosperity.

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Literature Cited

Abramoff, M., P. Magelhaes, and S. Ram. 2004. Image processing with ImageJ. Biophotonics International **11**:36-42.

Ali, J. G., and A. A. Agrawal. 2014. Asymmetry of plant-mediated interactions between

specialist aphids and caterpillars on two milkweeds. Functional Ecology **28**:1404-1412.

Appel, H. M., H. Fescemyer, J. Ehlting, D. Weston, E. Rehrig, T. Joshi, D. Xu, J. Bohlmann, and J. Schultz. 2014. Transcriptional responses of *Arabidopsis thaliana* to chewing and sucking insect herbivores. Frontiers in Plant Science **5**:565.

Babst, B. A., R. A. Ferrieri, D. W. Gray, M. Lerdau, D. J. Schlyer, M. Schueller, M. R. Thorpe, and C. M. Orians. 2005. Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. New Phytologist **167**:63-72.

Babst, B. A., R. A. Ferrieri, M. R. Thorpe, and C. M. Orians. 2008. *Lymantria dispar* herbivory induces rapid changes in carbon transport and partitioning in *Populus nigra*. Entomologia Experimentalis et Applicata **128**:117-125.

Barton, Kasey E., and J. Koricheva. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. The American Naturalist **175**:481-493.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B **57**:289-300.

Boege, K., and R. Marquis. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends in Ecology & Evolution **20**:441-448.

Boyd, I. L., P. H. Freer-Smith, C. A. Gilligan, and H. C. J. Godfray. 2013. The consequence of tree pests and diseases for ecosystem services. Science **342**:823-832.

Butin, E., E. Preisser, and J. Elkinton. 2007. Factors affecting settlement rate of the hemlock woolly adelgid, *Adelges tsugae*, on eastern hemlock, *Tsuga canadensis*. Agricultural and Forest Entomology **9**:215-219.

Coley, P., J. Bryant, and F. Chapin. 1985. Resource availability and plant antiherbivore

438 defense. Science **230**:895-899.

439 Delauney, A. J., and D. P. S. Verma. 1993. Proline biosynthesis and osmoregulation in
440 plants. Plant Journal **4**:215-223.

441 Denno, R., M. McClure, and J. Ott. 1995. Interspecific interactions in phytophagous
442 insects: competition reexamined and resurrected. Annual Review of Entomology **40**:297-331.

443 Domec, J.-C., L. N. Rivera, J. S. King, I. Peszlen, F. Hain, B. Smith, and J. Frampton.
444 2013. Hemlock woolly adelgid (*Adelges tsugae*) infestation affects water and carbon relations of
445 eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*). New
446 Phytologist **199**:452-463.

447 Ellison, A., M. Bank, B. Clinton, E. Colburn, K. Elliott, C. Ford, D. Foster, B. Kloeppel,
448 J. Knoepp, G. Lovett, J. Mohan, D. Orwig, N. Rodenhouse, W. Sobczak, K. Stinson, J. Stone, C.
449 Swan, J. Thompson, B. Von Holle, and J. Webster. 2005. Loss of foundation species:
450 consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and
451 the Environment **3**:479-486.

452 Erb, M., V. Flors, D. Karlen, E. de Lange, C. Planchamp, M. D'Alessandro, T. C. J.
453 Turlings, and J. Ton. 2009. Signal signature of aboveground-induced resistance upon
454 belowground herbivory in maize. Plant Journal **59**:292-302.

455 Fournier, V., J. A. Rosenheim, J. Brodeur, J. M. Diez, and M. W. Johnson. 2006.
456 Multiple plant exploiters on a shared host: Testing for nonadditive effects on plant performance.
457 Ecological Applications **16**:2382-2398.

458 Gandhi, K., and D. Herms. 2010. Direct and indirect effects of alien insect herbivores on
459 ecological processes and interactions in forests of eastern North America. Biological Invasions
460 **12**:389-405.

Gomez, S., L. Gonda-King, C. M. Orians, D. A. Orwig, R. Panko, L. Radville, N. Soltis, C. S. Thornber, and E. L. Preisser. 2015. Interactions between invasive herbivores and their long-term impact on New England hemlock forests. Biological Invasions **17**:661-673.

Gómez, S., L. Gonda-King, C. M. Orians, D. A. Orwig, R. Panko, L. Radville, N. Soltis, C. S. Thornber, and E. L. Preisser. 2015. Interactions between invasive herbivores and their long-term impact on New England hemlock forests. Biological Invasions **17**:661-673.

Gómez, S., C. Orians, and E. Preisser. 2012. Exotic herbivores on a shared native host: tissue quality after individual, simultaneous, and sequential attack. Oecologia **169**:1015-1024.

Hadley, J. L., and J. L. Schedlbauer. 2002. Carbon exchange of an old-growth eastern hemlock (Tsuga canadensis) forest in central New England. Tree Physiology **22**:1079-1092.

Hillwig, M. S., M. Chiozza, C. L. Casteel, S. T. Lau, J. Hohenstein, E. Hernandez, G. Jander, and G. C. MacIntosh. 2016. Abscisic acid deficiency increases defence responses against *Myzus persicae* in *Arabidopsis*. Molecular Plant Pathology **17**:225-235.

Inbar, M., H. Doostdar, G. Leibee, and R. Mayer. 1999. The role of plant rapidly induced responses in asymmetric interspecific interactions among insect herbivores. Journal of Chemical Ecology **25**:1961-1979.

Lee, S. C., and S. Luan. 2012. ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant Cell and Environment **35**:53-60.

Lieurance, D., S. Chakraborty, S. R. Whitehead, J. R. Powell, P. Bonello, M. D. Bowers, and D. Cipollini. 2015. Comparative herbivory rates and secondary metabolite profiles in the leaves of native and non-native *Lonicera* species. Journal of Chemical Ecology **41**:1069-1079.

Lovett, G. M., C. D. Canham, M. A. Arthur, K. C. Weathers, and R. D. Fitzhugh. 2006. Forest ecosystem responses to exotic pests and pathogens in eastern North America. Bioscience

56:395-405.

McAvoy, T. J., J. Régnière, R. St-Amant, N. F. Schneeberger, and S. M. Salom. 2017. Mortality and recovery of hemlock woolly adelgid (*Adelges tsugae*) in response to winter temperatures and predictions for the future. Forests **8**: 497.

McClure, M. 1980. Competition between exotic species: scale insects on hemlock. Ecology **61**:1391-1401.

McClure, M. 1989. Importance of weather to the distribution and abundance of introduced adelgid and scale insects. Agricultural & Forest Meteorology **47**:291-302.

Miller-Pierce, M., D. Orwig, and E. Preisser. 2010. Effects of hemlock woolly adelgid and elongate hemlock scale on eastern hemlock growth and foliar chemistry. Environmental Entomology **39**:513-519.

Morris, W. F., R. A. Hufbauer, A. A. Agrawal, J. D. Bever, V. A. Borowicz, G. S. Gilbert, J. L. Maron, C. E. Mitchell, I. M. Parker, A. G. Power, M. E. Torchin, and D. P. Vazquez. 2007. Direct and interactive effects of enemies and mutualists on plant performance: A meta-analysis. Ecology **88**:1021-1029.

Nykänen, H., and J. Koricheva. 2004. Damage-induced changes in woody plants and their effects on insect herbivore performance: A meta-analysis. Oikos **104**:247-268.

Oksanen, J., F. Blanchet, R. Kindt, P. Legendre, P. Minchin, R. O'Hara, G. Simpson, P. Solymos, M. Stevens, and H. Wagner. 2013. vegan: Community Ecology Package.

Oten, K. L. F., A. C. Cohen, and F. P. Hain. 2014. Stylet bundle morphology and trophically-related enzymes of the hemlock woolly adelgid (Hemiptera: Adelgidae). Annals of the Entomological Society of America **107**:680-690.

Pieterse, C. M. J., and M. Dicke. 2007. Plant interactions with microbes and insects: from

507 molecular mechanisms to ecology. Trends in Plant Science **12**:564-569.

508 Pinheiro, J., D. Bates, S. DebRoy, and D. Sarkar. 2014. R Core Team, 2014. nlme: Linear
509 and nonlinear mixed-effects models. R package version 3.1-118.
510 <http://cran.rproject.org/package=nlme>.

511 Polgar, C. A., and R. B. Primack. 2011. Leaf-out phenology of temperate woody plants:
512 from trees to ecosystems. New Phytologist **191**:926-941.

513 Preisser, E., and J. Elkinton. 2008. Exploitative competition between invasive herbivores
514 benefits a native host plant. Ecology **89**:2671-2677.

515 Preisser, E., A. Lodge, D. Orwig, and J. Elkinton. 2008. Range expansion and population
516 dynamics of co-occurring invasive herbivores. Biological Invasions **10**:201-213.

517 Radville, L., A. Chaves, and E. Preisser. 2011. Variation in plant defense against invasive
518 herbivores: evidence for a hypersensitive response in eastern hemlocks (*Tsuga canadensis*).
519 Journal of Chemical Ecology **37**:592-597.

520 Rauschert, E. S. J., and K. Shea. 2012. Invasional interference due to similar inter- and
521 intraspecific competition between invaders may affect management. Ecological Applications
522 **22**:1413-1420.

523 RCoreTeam. 2014. R: a language and environment for statistical computing. R
524 Foundation for Statistical Computing.

525 Rogers, B. M., P. Jantz, and S. J. Goetz. 2017. Vulnerability of eastern US tree species to
526 climate change. Global Change Biology **23**:3302-3320.

527 Schaeffer, R. N., C. M. Wilson, L. Radville, M. Barrett, E. Whitney, S. Roitman, E. R.
528 Miller, B. E. Wolfe, C. S. Thornber, C. M. Orians, and E. L. Preisser. 2017. Individual and non-
529 additive effects of exotic sap-feeders on root functional and mycorrhizal traits of a shared conifer

host. Functional Ecology **31**:2024-2033.

Schliep, E. M., N. K. Lany, P. L. Zarnetske, R. N. Schaeffer, C. M. Orians, D. A. Orwig, and E. L. Preisser. 2018. Joint species distribution modelling for spatio-temporal occurrence and ordinal abundance data. Global Ecology and Biogeography **27**:142-155.

Simberloff, D., and B. Von Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biological Invasions **1**:21-32.

Soler, R., F. R. Badenes-Perez, C. Broekgaarden, S. J. Zheng, A. David, W. Boland, and M. Dicke. 2012. Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: From insect performance to gene transcription. Functional Ecology **26**:156-166.

Soler, R., T. M. Bezemer, A. M. Cortesero, W. H. Van der Putten, L. E. M. Vet, and J. A. Harvey. 2007. Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. Oecologia **152**:257-264.

Soltis, N. E., S. Gómez, L. Gonda-King, E. L. Preisser, and C. M. Orians. 2015. Contrasting effects of two exotic invasive hemipterans on whole-plant resource allocation in a declining conifer. Entomologia Experimentalis et Applicata **157**:86-97.

Soltis, N. E., S. Gomez, G. G. Leisk, P. Sherwood, E. L. Preisser, P. Bonello, and C. M. Orians. 2014. Failure under stress: the effect of the exotic herbivore *Adelges tsugae* on biomechanics of *Tsuga canadensis*. Annals of Botany **113**:721-730.

Stam, J. M., A. Kroes, Y. H. Li, R. Gols, J. J. A. van Loon, E. H. Poelman, and M. Dicke. 2014. Plant interactions with multiple insect herbivores: from community to genes. Annual Review of Plant Biology **65**:689-713.

Trumble, J., D. Kolodny-Hirsch, and I. Ting. 1993. Plant compensation for arthropod

553 herbivory. Annual Review of Entomology **38**:93-119.

554 van Zandt, P., and A. Agrawal. 2004. Community-wide impacts of herbivore-induced
555 plant responses to milkweed (*Asclepias syriaca*). Ecology **85**:2616-2629.

556 Viswanathan, D., A. Narwani, and J. Thaler. 2005. Specificity in induced plant responses
557 shapes patterns of herbivore occurrence on *Solanum dulcamara*. Ecology **86**:886-896.

558 Vos, I. A., A. Verhage, R. C. Schuurink, L. G. Watt, C. M. J. Pieterse, and S. C. M. Van
559 Wees. 2013. Onset of herbivore-induced resistance in systemic tissue primed for jasmonate-
560 dependent defenses is activated by abscisic acid. Frontiers in Plant Science **4**:539.

561 Wallin, K. F., and K. F. Raffa. 2001. Effects of folivory on subcortical plant defenses:
562 Can defense theories predict interguild processes? Ecology **82**:1387-1400.

563 Yang, S. A., M. J. Ferrari, and K. Shea. 2011. Pollinator behavior mediates negative
564 interactions between two congeneric invasive plant species. American Naturalist **177**:110-118.

565 Zhou, S. Q., Y. R. Lou, V. Tzin, and G. Jander. 2015. Alteration of plant primary
566 metabolism in response to insect herbivory. Plant Physiology **169**:1488-1498.

567 Zvereva, E., V. Lanta, and M. Kozlov. 2010. Effects of sap-feeding insect herbivores on
568 growth and reproduction of woody plants: a meta-analysis of experimental studies. Oecologia
569 **163**:949-960.

570

571 **Table 1:** Treatments are arranged in a 3 x 3 full-factorial design, with years of infestation
572 by both hemlock woolly adelgid (HWA) and elongate hemlock scale (EHS) indicated. Numbers
573 in parentheses indicate the number of replicates for each treatment. Insect densities (mean ± 1 SE
574 insects/cm branch) were measured in November 2014.
575

		HWA presence		
		0 years of HWA	*2 years of HWA*	*4 years of HWA*
EHS presence	*0 years of EHS*	(12) **Control** EHS = 0 HWA = 0	(10) **HWA-2** EHS = 0 HWA = 2.36 ± 0.31	(13) **HWA-4** EHS = 0 HWA = 1.74 ± 0.20
	2 years of EHS	(9) **EHS-2** EHS = 1.79 ± 0.37 HWA = 0	(7) **Both-2** EHS = 1.64 ± 0.42 HWA = 1.11 ± 0.22	(9) **HWA → Both** EHS = 0.83 ± 0.26 HWA = 1.29 ± 0.27
	4 years of EHS	(9) **EHS-4** EHS = 2.19 ± 0.37 HWA = 0	(12) **EHS → Both** EHS = 2.10 ± 0.72 HWA = 1.37 ± 0.33	(6) **Both-4** EHS = 1.11 ± 0.18 HWA = 1.21 ± 0.24

576

577 **Table 2**: A) Mean amino acids (μg/g dry tissue) from A) one-year needles and B) >1- year

578 needles, by treatment and ranked in order of significance.

579 A.

				Amino acid concentrations (μg g^{-1} DM ± SE)		
Amino Acid	*F*-value	Rank	B-H *P*-value	0 years HWA	2 years HWA	4 years HWA
VAL	34.45	1	0.007	23.8 (1.3)	15.7 (1.7)	10.4 (1.1)
PRO	32.59	2	0.014	234.6 (22.3)	663.1 (71.1)	574.5 (48.5)
ILE	26.46	3	0.020	12.8 (0.8)	9.7 (2.2)	5.2 (0.7)
TRP	19.17	4	0.027	29.1 (1.7)	23.1 (1.5)	19.1 (1.3)
LYS	15.22	5	0.034	0.11 (0.011)	0.08(0.010)	0.05 (0.010)
THR	15.12	6	0.041	15.1 (1.0)	13.9 (0.8)	10.8 (1.0)
SER	13.48	7	0.048	202.1 (20.6)	207.9 (19.6)	143.4 (10.9)

580

581 B.

				Amino acid concentrations (μg g^{-1} DM ± SE)		
Amino Acid	*F*-value	Rank	B-H *P*-value	0 years HWA	2 years HWA	4 years HWA
PRO	21.27	1	0.007	199.4 (18.8)	417.5 (33.7)	370.1 (32.6)
VAL	14.02	2	0.014	18.5 (1.8)	13.1 (1.7)	9.3 (1.0)
TRP	13.15	3	0.021	18.3 (0.9)	16.1 (0.4)	14.5 (0.8)
ILE	11.28	4	0.029	13.5 (3.3)	7.3 (0.9)	4.7 (0.6)
THR	11.11	5	0.036	10.8 (0.8)	10.3 (1.2)	7.3 (0.8)
SER	5.65	6	0.043	140.8 (14.8)	152.2 (17.1)	120.6 (12.6)
LEU	4.56	7	0.050	3.4 (0.6)	2.6 (0.5)	2.0 (0.3)

582

583

584

585 **Figure Legends**

586 **Figure 1.** Ratio of (A, B) above- to below-ground biomass and (C, D) needle to wood
587 biomass in response to attack by hemlock woolly adelgid (HWA) (A, C) and elongate hemlock
588 scale (EHS) (B, D) following zero, two, or four years of infestation. Bars represent means ± 1
589 SE. Letters indicate a significant difference among groups based on a post-hoc Tukey HSD test.

590 **Figure 2.** Mean ± 1 SE rate of new foliage production (grams/day) in early spring
591 following zero, two, and four years of infestation by (A) hemlock woolly adelgid (HWA) and (B)
592 elongate hemlock scale (EHS). Letters indicate a significant difference among groups based on a
593 post-hoc Tukey test.

594 **Figure 3.** Mean ± 1 SE of (A) photosynthetic rate, (B) percent nitrogen, (C) total starch
595 concentration, and (D) total amino acids across different tissue types (new flush, 1-yr needles,
596 >1-yr needles). Length of hemlock woolly adelgid (HWA) infestation spans zero years (light
597 orange), two years (medium orange), and four years (red). Letters indicate a significant
598 difference among groups based on post-hoc Tukey tests, while n.d. indicates a lack of data for
599 that tissue class and/or trait measurement.

600 **Figure 4.** Non-metric multidimensional scaling (NMDS) plots of amino acid profiles for
601 A) 1-year and B) >1-year needles following zero (yellow), two (orange), and four (red) years of
602 hemlock woolly adelgid (HWA) infestation. Symbols denote mean values; lines through symbols
603 denote standard errors.

604